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Platelet and Red Cell Indices of anti-Dengue IgM Negative Blood Donors in Owo, Southwest NigeriaMuhibi, M.A.^{1,2*}, Agada, P.², Ifeanyichukwu, M.³, Jeremiah, A.Z.⁴

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Abstract

Dengue affects an estimated 50 million people in approximately 100 countries annually and is one of the causative agents of viral haemorrhagic fever, which is transmitted primarily through *Aedes* mosquitoes and parenterally. The aim was to determine the incidence of Dengue virus infection among healthy blood donors in Owo, Southwest Nigeria and correlate the fundamental blood cells indices with Dengue infection. A total of 150 healthy blood donors were examined for presence of anti-dengue antibodies (IgM) to establish ongoing or recent dengue infection. Enzyme linked immunosorbent assay (ELISA) kit was used to assay for anti-Dengue IgM antibodies in the sera of 150 participants and their EDTA anticoagulated blood specimen were subjected to flow cytometry using Sysmex autoanalyser to quantify WBC, PLT, MPV, PDW, P-LCR, HGB, HCT, MCV, MCH and MCHC. Albeit, all participants tested negative to Dengue virus infection. Results obtained for WBC, PLT, MPV, PDW, P-LCR, HGB, HCT, MCV, MCH and MCHC were 6.62 ± 3.91 , 212.23 ± 113.34 , 11.56 ± 8.90 , 15.13 ± 12.40 , 34.90 ± 32.71 , 12.30 ± 2.06 , 39.03 ± 5.77 , 82.56 ± 9.05 , 26.52 ± 2.57 and 31.70 ± 1.43 respectively. All participants who were adults tested negative and the haematological indices of the participants were normal. A reference range for the haematological indices among adults in Owo could be deduced from this work, while efforts should be put in place to sustain surveillance of neglected tropical diseases that are transmissible through blood transfusion for possible review of blood safety programmes in the region.

Keywords: Dengue infection, reference range, haematological parameters, blood safety, neglected tropical diseases.

Introduction

Viral haemorrhagic fevers are infectious diseases that interfere with the blood's ability to clot, damage the walls of blood vessels, and are caused by infection with viruses of which Dengue virus is one (WHO, 2009). Recent studies have affirmed the continued transmission of the infection in Nigeria (Faneye *et al.*, 2013; Baba *et al.*, 2009) and presence of immunoglobulin M and G specific for the virus in south western Nigeria adults (Muhibi *et al.*, 2017; Adeleke *et al.*, 2013). The persistent transmission of the virus in Nigeria could be premised on the prolific breeding and predominance of *Aedes aegypti* and the invading *Aedes albopictus* and by the cross-border travelers from endemic countries (mostly Asian and American continents) to Nigeria (Adeleke *et al.*, 2013; Mbanugo and Okpalanouju, 2003). Being a virus that is equally transmissible parentally (Chen and Wilson, 2004) and with potentials to exhibit asymptomatic phase in the infected host (Muhibi *et al.*, 2017; Ngo *et al.*, 2001), it is of concern in blood transfusion safety. The literature revealed that the prevalence of dengue virus varies from one population to another and constitute a source of concern in tropical countries, Nigeria inclusive (Chunsuttiwat *et al.*, 2005). It is also reported that dengue virus was transmitted vertically and through bone marrow transplantation (Chen and Wilson, 2005), although the celebrated route is via bites of aedes mosquitoes (Halstead, 2005). The aim of this work was to determine the

Dengue virus infection among blood donors in Owo; using immunoglobulin M as the marker and correlate the positivity to the platelet and red cell indices.

Materials and Methods

Study subjects: A total of 150 apparently healthy blood donors from Federal Medical Center, Owo who consented to participate in the study were recruited. All the blood donors were offered pre-test and post-test counseling.

Procedure:

This study was carried out at Federal Medical Center, Owo in Southwestern Nigeria. Owo has an estimated population of 276,593 (NPC, 2006) and it is located within the low rain forest zone of Nigeria with two seasons, dry and wet. The primary occupation of Owo people is farming and trading. Five milliliters of venous blood were collected separately from each subject by vene-puncture into plain and EDTA vacutainer tubes for Dengue virus serology and haematological indices respectively. The clotted specimens were centrifuged at 1200 revolution per minute (rpm) for 5 minutes to harvest serum into a micro-tube for the anti-Dengue (**IgM**) testing with third generation Enzyme Immuno-Assay (EIA) method. The kit was manufactured by DIAPRO Diagnostics (Italy). Donors' sera were diluted 1 in 100 and dispensed to the microwells alongside the undiluted positive and negative controls. The microwells were washed 3 times after 60 minutes incubation at 37°C, then the enzyme horseradish peroxidase (the HRP-Conjugate) was added before the second incubation. Chromogen/substrate mixture was added to every well except blank and the microplate was incubated at room temperature in the dark for 20 minutes. Sulphoric Acid was eventually added to every microwell to stop the enzymatic reaction. During incubation, the specific immunocomplex formed in case of presence of anti-Dengue (IgM) in the sample, was captured on the solid phase which generates an optical signal that is proportional to the amount of anti-Dengue antibodies present in the sample. The commercially prepared positive and negative controls were treated alongside the specimens. A cut-off value was determined and

results were interpreted as positive and negative according to manufacturer's instructions. The EDTA samples were subjected to KX-21N Sysmex haematology analyser to generate the red cell and platelet indices. The standard operating procedures for the determination of haematological parameters using sysmex KX-21N auto haematology parameters were adhered to. Parameters determined include; Red Blood Cell Count (RBC), Haemoglobin (HGB), Haematocrit (HCT), Mean Cell Volume (MCV), Mean Cell Haemoglobin (MCH), Mean Cell Haemoglobin Concentration (MCHC), Platelets (PLT), Mean Platelet Volume (MPV), Platelet Distribution Width (PDW) and P-LCR.

Statistical Analysis

The data generated were coded, entered, validated and analyzed using Statistical Package for Social Science (SPSS) version 22. The seroprevalence of anti- Dengue IgM antibody was expressed for the entire study group and documentation of participants' age, gender, knowledge and attitude was done. The measures of central tendencies, dispersions and variations were also expressed for the parameters accordingly. A p-value of ≤ 0.05 was considered significant in all statistical analysis.

Results

Out of the 150 voluntary blood donors recruited for Dengue virus IgM screening, 104 (69.33%) were in the active age group (20–40 years) accounting for the majority of the participants. None of the participant was older than 60. In terms of gender distribution, men (68.7%) were in the majority. Marital profile showed that most of the participants were married (75.33%). Distribution based of employment status indicated that majority of the subjects were civil servants 116(77.33 %) (Table 1). The results of the seroprevalence of IgM antibodies revealed that none of the donors was positive. The white blood cells count, absolute platelet count, MPV, PDW, P-LCR, HGB, HCT, MCV, MCH and MCHC obtained by automated flow cytometry were 6.62 ± 3.91 , 212.23 ± 113.34 , 11.56 ± 8.90 , 15.13 ± 12.40 , 34.90 ± 32.71 , 12.30 ± 2.06 , 39.03 ± 5.77 , 82.56 ± 9.05 , 26.52 ± 2.57 and 31.70 ± 1.43 respectively (Table 2).

Table 1: Socio-demographic variables of the blood donors in Owo

Variables	Frequency	Percentage
Age Group		
<20 years	06	4.0
21-30 years	53	35.33
31-40 years	51	34.0
41-50 years	26	17.33
51-60 years	14	9.33
Gender		
Male	103	68.7
Female	47	31.3
Marital status		
Single	36	24.0
Married	113	75.33
Widow	01	0.66
Employment Status		
Artisans	33	22.0
Civil servants	116	77.33
Unemployed	1	0.66

Table 2: Some haematological parameters of blood donors in Owo

Haematological Parameter	N	Minimum	Maximum	Mean	Std. Deviation
WBC	150	2.90	43.30	6.62	3.91
PLT	150	58.00	1136.00	212.23	113.34
MPV	150	8.10	119.00	11.56	8.90
PDW	150	7.90	163.00	15.13	12.40
PLCR	150	10.40	334.60	34.90	32.71
Hb	150	4.65	16.20	12.30	2.06
PCV	150	19.60	49.60	39.03	5.77
MCV	150	29.60	111.70	82.56	9.05
MCH	150	13.50	36.20	26.52	2.57
MCHC	150	23.60	35.60	31.70	1.43

Discussion

One hundred and fifty apparently healthy blood donors were examined for presence of anti-Dengue IgM antibodies and their blood cells parameters were assayed to determine the correlation, if any, between the parameters and infection with Dengue virus. The age range of

subjects was 18-60 years. All donors recruited in this study tested negative to anti-Dengue (IgM) ELISA test (Prevalence= 0%). The haematological indices of the participants were normal and reflect what could be useful as reference range for the parameters in Owo, as against the use of reference range from other

climes. Dengue infection prevalence varies from one setting to another and accuracy of reports on its prevalence depends on specificity and sensitivity of technology employed in each research work.

Dengue-like illness has been recorded in Africa though usually without laboratory confirmation and could be due to infection with dengue virus or with viruses such as Chikungunya that produce similar clinical symptoms or in co-infection with malaria or typhoid (Baba *et al.*, 2013).

Fagbami *et al.* (1977) investigated the prevalence of flavivirus infections among 1,816 children and adults from urban and rural areas in samples obtained mainly during the early 1970s in Nigeria. Dengue virus-specific haemagglutination inhibition testing showed that the prevalence of immunity was 38% for DENV-1 infection and 45% for DENV-2 infection. The authors also reported proportional increase in prevalence of DENV with age, which suggests the endemicity of the infection in Nigeria. Collenberg *et al.* (2006) reported that the prevalence of antibodies against DENV determined by immunoglobulin G indirect ELISA among a sample of pregnant women and blood donors (n = 683) was 26.3% in rural settings and 36.5% in urban settings in Burkina Faso. In Cameroon, the prevalence of antibodies against DENV determined by neutralization testing among 256 adults was found to be 12.5% (Kuniholm, *et al.*, 2006).

Conclusion

In conclusion, the results of this study have highlighted Dengue virus infection is not common in Owo. Samples of apparently healthy individuals of age groups from infancy to geriatrics should be tested for blood cells parameters to determine the reference ranges for the stratified populations in Owo, southwest Nigeria.

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